Bioequivalence Assessment Of Topical Clobetasol Propionate Products Using Visual And Chromametric Assessment Of Skin Blanching

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ABSTRACT – PURPOSE. The assessment of the degree of skin blanching following the application of a formulation containing a topical corticosteroid has been established as a surrogate method for the determination of bioequivalence. In this study, both visual and chromametric assessments have been carried out on two topical creams containing clobetasol propionate (0.05%) and the results from both methods are compared. **METHODS.** Human subjects (volunteers) were screened using a cream containing 0.05% clobetasol propionate, in order to identify appropriate subjects for inclusion in the study. The study was implemented according to the FDA guidance using both visual and chromameter assessment techniques. Blanching responses were assessed visually by three trained, independent observers and instrumentally using a Chromameter®. An ED_{50} of 36 min was used as the dose duration based upon data previously obtained from a pilot study using the same topical corticosteroid reference product. A visual rating scale of 0–4 and the a-scale readings from the chromameter were used. **RESULTS.** The visual and chromameter blanching profiles showed similar blanching responses with good correspondence. The 90% confidence intervals for the data from both methods were calculated using Locke's method. When only the data obtained from 23 subjects who were identified as "detectors" (as per FDA guidance) were used, the products fell within the bioequivalence acceptance range of 80-125% using the visual assessment method (99.3-111.6%) whereas the data using a chromameter (86.5-129.3%) were just outside the acceptance limits. However, when all subjects (n=34) were included in the calculations, both the visual (97.9-109.2) and chromameter (90.2-120.7) data fell within the bioequivalence acceptance range. **CONCLUSIONS.** Whereas visual data indicated bioequivalence using either data from "detectors" or data from all subjects, the chromameter data from "detectors" only indicated bioinequivalence but inclusion of all subject data fell within the acceptance range to be declared bioequivalent.

INTRODUCTION

Topical corticosteroid preparations have been extremely effective for the treatment of various skin disorders such as eczema, psoriasis and keloids, amongst others (1-3). Following the expiry of patents on many topical corticosteroid products, multisource preparations have been developed and the bioequivalence assessment of such products compared to the innovator product has been a pre-requisite for market approval by regulatory authorities. The unique property of topical corticosteroids that induces skin whitening or blanching at the site of application has been used as a criterion to determine the bioavailability of topical corticosteroids formulated as a topical preparation. This provides a valuable tool for the assessment of bioequivalence of products containing topical corticosteroids. This procedure, known as the "Human Skin Blanching Assay" (HSBA) is currently the accepted method for bioequivalence assessment of such topical formulations. It has also been used for potency ranking of topical corticosteroids (4, 5). A typical skin blanching response is illustrated by the whitening effect of the skin by the corticosteroid as shown in Figure 1.

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This assay was introduced by McKenzie and Stoughton (6) and involves the evaluation of the degree of skin blanching over a period following the application of topical corticosteroid products to the skin of healthy human subjects. It can be carried out by visual assessment of the degree of blanching at the application site or by an instrumental method of assessment using a chromameter. The intensity of the skin blanching response is related to the amount of corticosteroid that has penetrated into the skin (7). Various publications have shown that the precision, sensitivity and repeatability of this assay are adequate for the assessment of the bioequivalence of topical corticosteroid formulations (8-13). Recently, studies in yellow-skinned subjects have been reported which have demonstrated that the HSBA can be successfully used on both Chinese (14) and Japanese skin (15). However, the latter report indicated that although AUEC and ED_{50} could be determined on yellow-skinned races, negligible differences were found regarding dose duration reproducibility and seasonal changes. Studies have also shown that the composition of the vehicle can have a significant effect on the percutaneous absorption of topical corticosteroid. Formulation differences between test and reference product can therefore result in significant differences in bioavailability, which are demonstrated as differences in blanching between the products with associated implications for bioequivalence (16-17).

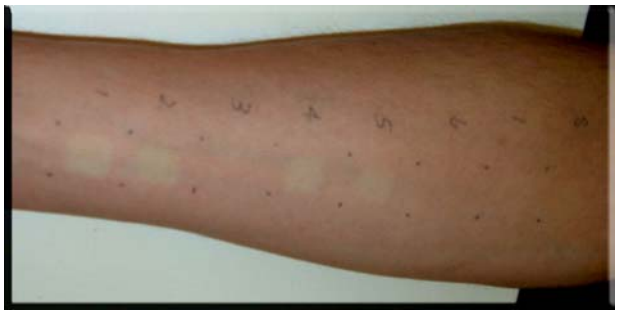

Figure 1. A typical skin blanching response

Many regulatory authorities, including the United States Food & Drug Administration (FDA) have adopted this type of study for the assessment of the bioavailability and bioequivalence of topical corticosteroid formulations. Currently, the FDA recommends that the degree of blanching be assessed preferably using a chromameter and/or by visual assessment (5). Several reports have been published on the use of these methods (18-19) and whilst the chromameter is currently perceived to be the method of choice (5), claims that the use of the visual assessment technique is more accurate have been reported (20-23). The visual method involves subjective assessment of the intensity of blanching at the application site in comparison to surrounding untreated skin. It also requires considerable training and subsequent use of experienced observers and is difficult if not impossible to standardize and validate. An instrumental method largely overcomes the limitations of subjective assessment by providing objective measurements from a calibrated instrument, which can be validated by establishing the reproducibility of measurements. However, it should be noted that training and experience in the use of a chromameter is essential to obtain reproducible and reliable results (24-25). The present study was undertaken to compare and determine the effectiveness of the chromameter and the eye as an evaluating tool for the HSBA.

METHODS

Subjects

This investigation consisted of three trials conducted on separate days. The entire study utilized 34 volunteers of various skin types ranging from Fitzpatrick skin type I-V (26) (12 males, 22 females, age range 20-26 years). The volunteers were previously screened with a 0.05% clobetasol propionate cream and accepted into the study based on their ability to show an adequate blanching response (5). None of the volunteers had been treated with topical corticosteroids for at least 2 months prior to the trials. Written informed consent was obtained from each volunteer before the commencement of the study. The study protocol was approved by the departmental ethical standards committee which is a delegated sub-committee of the Rhodes University Ethical Standards Committee.

Experimental design

The study was performed in accordance with the FDA guidelines (5) using both visual and chromameter assessment methods. Eight sites were used per forearm and demarcated using a

pre-punched adhesive label template exposing a 1.1 x 1.1 cm square for application of the test and reference preparations. Two cream formulations (containing 0.05% m/m clobetasol propionate), a test (T) and reference (R) product, were utilized in this study. Twelve microlitres (equivalent to ~11 mg) of each cream were applied to the designated application sites on both ventral forearms of each subject.

In accordance with the FDA guidance, three different dose durations (ED_{50}, D_1 and D_2) were used. ED_{50} is the dose duration at which half the maximum blanching response is achieved, D_1 is the dose duration equal to half of ED_{50} and D_2 is the dose duration equal to double that of ED_{50}. Both the test and reference products were applied for 36 min (ED_{50}) to the relevant sites demarcated as T and R respectively, as shown in Figure 2 and used to assess bioequivalence. Only the reference product was applied to the sites demarcated as D_1 (18 min) and D_2 (72 min) for the determination of "detectors" amongst the volunteers. Two of the eight sites were used as controls (UNT). These dose durations were previously determined from a pilot study and all application sites were randomized amongst the different volunteers.

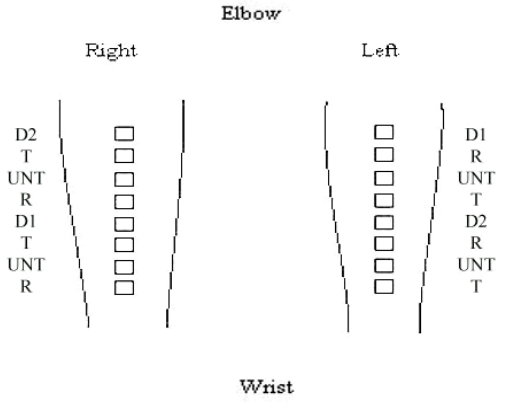

Figure 2. A schematic drawing of the application sites for the human skin blanching study.

Skin blanching was evaluated both visually and using a chromameter at 0, 2, 4, 6, 8, 10, 12, 15, 22, 25, and 30 hours after removal of the products by washing each application site in accordance with the FDA guideline (5). Standard lighting by overhead fluorescent lamps were used for all the studies. Room temperature (22 ± 2 ◦C) and humidity (55 ± 3 %) were controlled throughout the studies.

Visual assessment

The degree of pallor was estimated by three trained observers using a 0 – 4 point scale where 0 indicates no blanching and 4 indicates strong – intense blanching. Each site was assigned a blanching score by comparing the degree of blanching of the skin at the site of application to the surrounding, adjacent skin colour unaffected by the product. These data were presented as % TPS (percentage total possible score) which was calculated according to the method described by Haigh and Kanfer (27).

Chromameter assessment

A Minolta Chromameter® (Model CR 400, Minolta, Osaka, Japan) that provides measurements based on three scales, the L-scale, a-scale and b-scale, was used. In accordance with the FDA guidelines (5), only a-scale data were used to calculate the area under the effect curve (AUEC). The chromameter data were analyzed to determine which of the 'responders' were "detectors". The FDA guidance states that a "detector" is a 'responder' whose blanching data must meet the following criterion: AUEC at D_2 / AUEC at $D_1 \geq 1.25$ (5).

Data analysis

AUEC values for visual and chromameter data were determined using the trapezoidal rule. Statistical analysis was carried out using Locke's method (5) to determine bioequivalence of the formulations using data for all subjects (n=34) and data for "detectors" only (n=23).

RESULTS

Figures 3 and 4 represent the mean visual blanching profiles for the clobetasol propionate creams obtained from the groups of "detectors" and all the subjects, respectively. These profiles were plotted as % TPS (total possible score from three observers) versus time. Figures 5 and 6 depict the mean chromameter blanching profiles for the clobetasol propionate creams obtained from the groups of "detectors" and all the subjects, respectively. These two blanching response profiles were plotted as a-scale (multiplied by -1, to yield positive values) versus time.

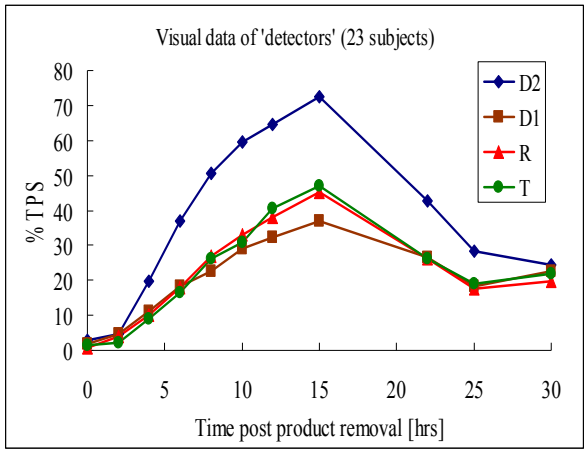

Figure 3. Mean visual blanching response profiles for the "detectors".

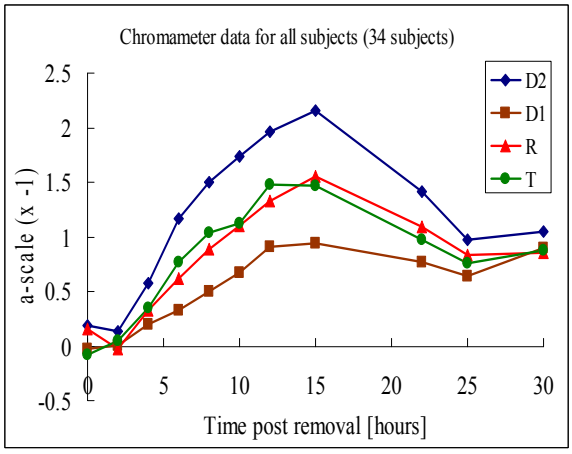

Figure 6. Mean chromameter blanching response profiles for all subjects.

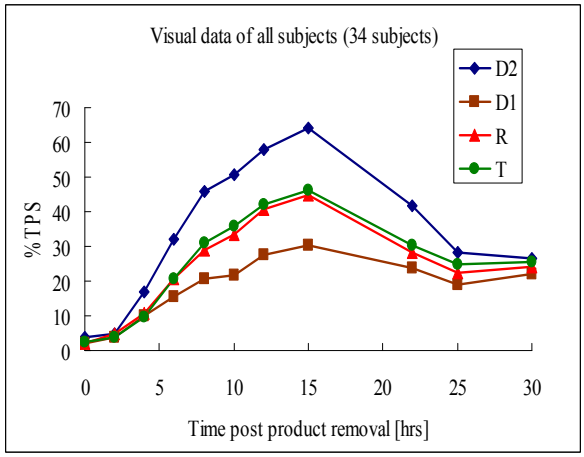

Figure 4. Mean visual blanching response profiles for all subjects

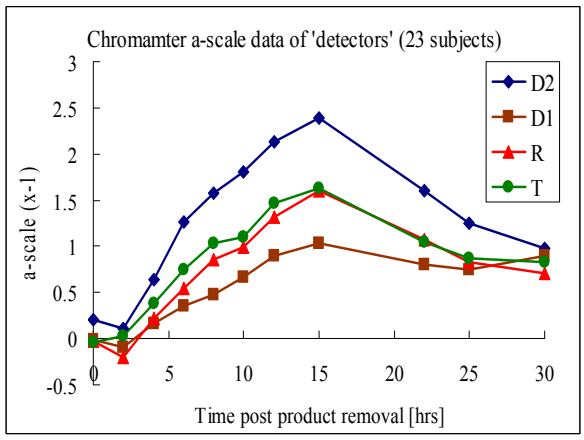

Figure 5. Mean chromameter blanching response profiles for "detectors"

The four graphs showed very similar blanching profiles and all the profiles illustrated that the blanching response peaked at 15 hours after product removal. Comparison of the profiles of "detectors" and of all the subjects revealed that the curves were quite similar to each other.

As seen from Table 2 below, the visual data obtained from "detectors" fell within the bioequivalence acceptance range of 80–125 % whereas the corresponding chromameter data slightly exceeded the upper limit of 125%. However, when the data from all subjects were used, both visual data and chromameter data were within the acceptance range. Overall, the visual data yielded a narrower interval compared to the chromameter data and using the data from all subjects yielded a narrower range than the using only "detectors" data.

DISCUSSION

The comparison of the blanching response profiles, as shown in Figures 3, 4, 5 and 6, are all quite similar, indicating that the exclusion of "non-detectors" appears to have very little effect on the overall profiles. It also indicates that the two techniques are equally applicable for the evaluation of blanching.

The acceptance criteria for bioequivalence of oral dosage forms must fall within the range of 80-125 % (28). However, whether this acceptance range should be applied for the assessment of bioequivalence of topical products is moot.

Table 1. AUECs for visual and chromameter

	VISUAL		CHROMAMETER*	
	Detectors (n=23)	All subjects (n=34)	Detectors (n=23)	All subjects (n=34)
TEST PRODUCT				
Mean AUEC	907.518	853.891	30.831	28.637
SD	453.674	431.579	16.138	16.715
CV%	50.0	50.5	52.3	58.4
REFERENCE PRODUCT				
Mean AUEC	891.803	829.534	28.302	27.460
SD	515.240	492.395	17.598	16.535
CV%	57.8	59.4	62.2	60.2

Table 2. 90% confidence intervals calculated using Locke's method for visual and chromameter data

	Visual		Chromameter	
	Mean Ratio % (T/R)	90% CI	Mean Ratio % (T/R)	90% CI
Detectors (n=23)	104.6	99.3 – 111.6	104.6	86.5 – 129.3
All Subjects (n=34)	102.9	97.9 – 109.2	104.3	90.2 – 120.7

In view of the relatively high variability (21-23) in percutaneous drug absorption amongst subjects and as reflected in the current data (Table 1) using visual or chromameter methods, consideration could be given to widening the acceptance range for the declaration of bioequivalence of topical formulations. Notwithstanding, blanching data from the 23 "detectors" in this study using the visual method and data from all the subjects using either visual or chromameter methods, resulted in the products falling within the bioequivalence acceptance interval. When, however, chromameter "detector" data were used, the products did not meet the criteria for the declaration of bioequivalence. It was however, interesting to note that the chromameter data yielded wider bioequivalence intervals than the corresponding data obtained from the visual method. Furthermore, these results clearly indicate that the eye is a reliable evaluating tool for the assessment of skin blanching. In addition, whereas the FDA guidance recommends that 40 to 60 evaluable subjects are required for a typical HSBA, data obtained from this study indicate that this number may not be necessary. Bioequivalence criteria were met with visual data using 23 "detectors" as well as data from all 34 subjects, where 90% CIs were similarly narrow for both sets. Furthermore, although the 90% CIs for chromameter data was wider for the 23 detectors, all subject data showed that 34 subjects were sufficient to demonstrate bioequivalence. This further supports our earlier suggestion that less than 40 evaluable subjects can be adequate.

CONCLUSIONS

Whilst, in general, regulatory agencies recommend the use of a chromameter for the assessment of skin blanching as opposed to visual assessment as the method of choice for determining bioequivalence of topical corticosteroid preparations, the results from this study clearly show the reliability and appropriateness of visual assessment. Furthermore, visual assessment confirmed the utility of this approach for the assessment of bioequivalence of topical clobetasol propionate preparations using only 23 "detectors" whereas 34 volunteers ("detectors" plus "non-detectors") were needed to obtain similar results to show bioequivalence using the chromameter to assess skin blanching.

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